Alzheimer's disease: **Clues from flies and worms**

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Presenilin mutations give rise to familial Alzheimer's disease and result in elevated production of amyloid β peptide. Recent evidence that presenilins act in developmental signalling pathways may be the key to understanding how senile plaques, neurofibrillary tangles and apoptosis are all biochemically linked.

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The classical pathological hallmarks of Alzheimer's disease are senile neuritic plaques and neurofibrillary tangles in the brain. Senile plaques consist of an extracellular core of deposited amyloid β peptide surrounded by degenerating neuronal processes. Neurofibrillary tangles are intraneuronal aggregates of paired helical filaments, which are assembled from hyperphosphorylated forms of the microtubule-associated protein tau. The dementia characteristic of Alzheimer's disease is the consequence of selective neuronal loss. Neurofibrillary tangles cause the death of neurons that contain them, but another cause of cell death may be direct toxicity of amyloid β peptide. There is evidence that at least some of the neuronal cell death in Alzheimer's disease is via apoptosis, but precisely how amyloid β peptide deposition, neurofibrillary tangle formation and the mechanism of cell death are linked has so far eluded researchers. But now clues to possible biochemical links between these processes are beginning to emerge as a result of recent genetic discoveries.

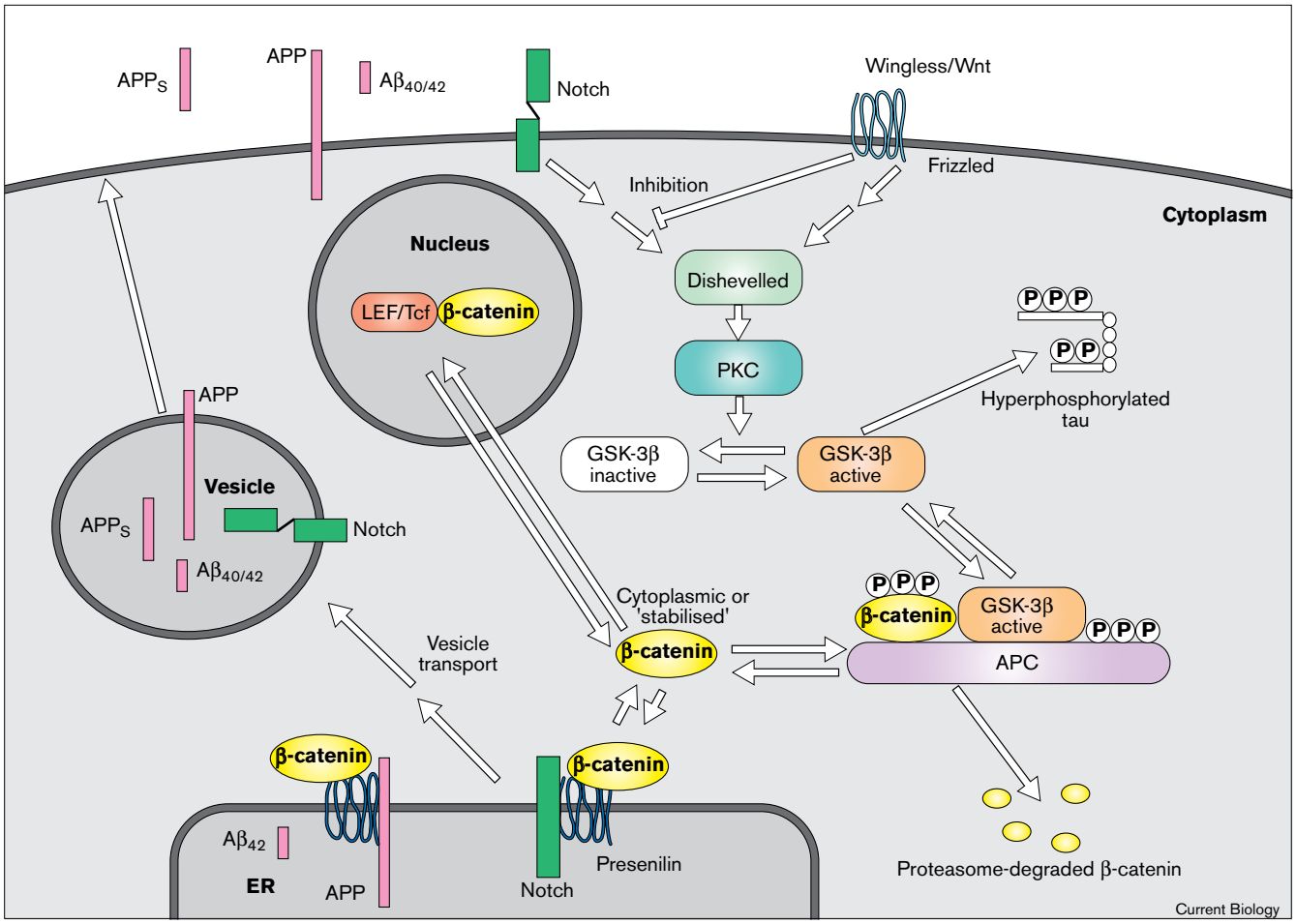
Most cases of autosomal dominant Alzheimer's disease are caused by mutations in the *presenilin-1* gene on chromosome 14, a variety of which (more than 50) have been described so far. In a few pedigrees, the disease is associated with mutations in the homologous gene *presenilin-2* on chromosome 1. Presenilin 1 and presenilin 2 are transmembrane proteins, probably with eight transmembrane domains, that are located primarily in the endoplasmic reticulum and Golgi apparatus. Several observations have indicated that presenilins play a part in signalling by Notch, which is known to have a number of important roles in development. Thus, both presenilins are homologous to Sel-12, a protein required for Notch signalling in the nematode *Caenorhabditis elegans*, possibly because they have a role in the processing and/or intracellular transport of Notch before it arrives at the plasma membrane [1]. And *presenilin-1* knockout mice die *in utero* with a phenotype similar to *Notch* mutants.

In addition to possibly having a developmental role in Notch signalling, presenilins in the brain have been shown to influence proteolytic processing of the amyloid precursor protein. Amyloid precursor protein is a single-pass transmembrane protein that is subject to several alternative proteolytic cleavages, including ones that release two forms of amyloid β peptide: one of 40 amino acids ($A\beta_{40}$) and the other of 42 amino acids ($A\beta_{42}$). The longer form $A\beta_{42}$ has a greater tendency to aggregate into fibrils, which have been shown to be neurotoxic, acting by induction of neuronal apoptosis. The expression in transgenic mice of a form of presenilin 1 carrying a mutation associated with autosomal dominant Alzheimer's disease was found to result in an increase in the $A\beta_{42}$:$A\beta_{40}$ ratio. As it seems that $A\beta_{42}$ is produced in the endoplasmic reticulum, and $A\beta_{40}$ in the Golgi apparatus, presenilins may influence amyloid β peptide production via an effect on the intracellular transport of amyloid precursor protein (Figure 1). Most researchers in the field have argued, on the basis of these observations, that mutations in the presenilin genes predispose individuals to Alzheimer's disease by promoting amyloid β peptide deposition in the brain, resulting in neuronal loss.

Several groups have recently shown that presenilins associate directly with β-catenin [2–6]. This is intriguing, because β-catenin is a component of the intracellular signal transduction pathway that mediates responses to the cell–cell signalling molecule Wingless/Wnt — *Drosophila*/vertebrate forms of the ligand, respectively — and there is genetic evidence in *Drosophila* that the Notch and Wingless pathways interact at the level of a protein called Dishevelled, or Dvl in vertebrates (Figure 1). Activation of the Wnt pathway by binding of extracellular Wnt-1 to its receptor, Frizzled, results in inhibition of glycogen synthase kinase-3β (GSK-3β) by an unknown mechanism involving Dvl and probably protein kinase C, which inactivates GSK-3β through phosphorylation [7].

GSK-3β phosphorylates 'adenomatous polyposis coli' protein (APC) and β-catenin, which both occur within cells in a complex with GSK-3β. Phosphorylated β-catenin is then ubiquitinated and degraded via the proteasome pathway. So when GSK-3β is inhibited as a result of activation of the Wnt pathway, the cytoplasmic levels of β-catenin rise, a response commonly referred to as 'β-catenin stabilisation'. This increase in its cytoplasmic level is accompanied by the translocation of some β-catenin to the nucleus, where it regulates gene expression through interaction with members of the Tcf/LEF family of transcription factors [7]. Although tau is a substrate for several protein

Figure 1



A hypothetical scheme by which Notch and Wingless/Wnt signalling might link the processing of amyloid precursor protein (APP) – to APP_S, $A\beta_{40}$ and $A\beta_{42}$ – and tau hyperphosphorylation. GSK-3β phosphorylates tau, and when the Notch/Wnt pathway is stimulated, GSK-3β is inhibited and β-catenin is 'stabilised' in the cytoplasm. As the cytoplasmic levels of β-catenin rise, some is translocated to the nucleus, where it interacts with transcription factors (LEF/Tcf). Presenilins regulate the production of amyloid β-peptide, some of which occurs in the endoplasmic reticulum (ER). Although it is unclear if presenilins physically interact with amyloid precursor protein or Notch, presenilins do appear to regulate processing and intracellular transport of amyloid precursor protein and Notch – potentially closing a regulatory loop that involves both amyloid precursor protein and tau.

kinases, GSK-3β is the best candidate for being the enzyme that generates the hyperphosphorylated tau that is characteristic of paired helical filaments [8]. So are the Notch and Wnt signalling pathways the keys to understanding Alzheimer's disease? Several recent papers suggest that they might be, but unfortunately not entirely in a consistent manner.

Zhang *et al.* [2] found that, in several cell types, presenilin 1 occurs in a complex with β-catenin, APC and GSK-3β. They also showed, by immunofluorescence microscopy, that β-catenin and presenilin 1 are co-localised in the Golgi apparatus. Some β-catenin was also localised at the plasma membrane — presumably complexed to cadherins — and some presenilin 1 was present in the endoplasmic reticulum. The obvious question to address was whether mutant presenilins could influence β-catenin metabolism and, especially, 'stabilisation'.

In their paper, Zhang *et al.* [2] imply that overexpression of wild-type presenilin 1 does result in stabilisation of β-catenin, and that mutant presenilin 1 does this less effectively. But they did not actually measure stabilisation — the levels of cytoplasmic β-catenin — but rather the turnover of total cellular β-catenin, both cytoplasmic and membrane-bound forms. They found that overexpression of wild-type presenilin 1 in transfected cells resulted in a slower turnover rate for β-catenin, as measured by pulse–chase labelling, and that the β-catenin turnover rate in cells making mutant presenilin 1 was higher than that in

cells making wild-type presenilin 1. Consistent with this, they found lowers levels of β-catenin in extracts of brain samples from Alzheimer's cases with presenilin 1 mutations compared to controls or sporadic Alzheimer's cases. And in fibroblasts from *presenilin 1* mutant mouse embryos they observed increased levels of β-catenin degradation products, indicating that presenilin 1 is required to maintain a basal physiological level of β-catenin.

In a further set of experiments, Zhang *et al.* [2] investigated whether β-catenin plays a role in apoptosis induced by amyloid β peptide. They found that primary rat hippocampal neurons were more vulnerable to apoptosis induction by amyloid β peptide when they were transfected with a construct encoding a dominant-negative mutant form of β-catenin. Co-transfection with a construct encoding wild-type β-catenin reversed the effect. A dominant-negative version of the Tcf transcription factor also rendered the neurons more vulnerable to apoptosis, both in the absence and presence of amyloid β peptide, and again this was reversed when the cells also made wild-type Tcf.

These observations may provide a mechanism explaining earlier findings in which expression of mutant presenilin 1 rendered cells more vulnerable to amyloid β peptide toxicity [9]. They also imply that possession of a mutant *presenilin* gene could be a 'doubly whammy' by both increasing $A\beta_{42}$ production and making neurons more vulnerable to amyloid β peptide toxicity. But as Zhang *et al.* [2] acknowledged, the complete opposite effect of β-catenin and Tcf on apoptosis has been reported [10]. In this latter study, it was found that reduced levels of β-catenin and expression of a dominant-negative mutant Tcf rescued cells from apoptosis. Zhang *et al.* [2] suggest the contrasting results may reflect the fact that different types of cells respond in different ways, but this seems a weak argument for such a ubiquitously expressed signalling pathway.

The paper from Zhang *et al.* [2] is certainly interesting and important, but it does not address the mechanism by which presenilins regulate β-catenin metabolism. The slowed β-catenin turnover as a result of presenilin overexpression, which is less in the case of mutant presenilins, might simply occur because there is a larger pool of a membrane-bound binding partner — presenilin 1 — for β-catenin, isolating it from the proteasome pathway. In *presenilin 1* mutant cells, the lack of such a binding partner could result in increased degradation, and Alzheimer's brain samples with one wild-type and one mutant *presenilin 1* allele may have reduced presenilin 1-binding capacity for β-catenin. A recent paper by Murayama *et al.* [3], however, does take us one step further towards a mechanism.

Murayama *et al.* [3] transiently over-expressed wild-type and mutant forms of presenilin 1 in COS-7 cells and assayed cytoplasmic β-catenin levels — thus they assayed true β-catenin stabilisation. They found that both wild-type and mutant forms of presenilin 1 reduced β-catenin stabilisation but did not affect the level of membrane-bound β-catenin. They also measured Tcf activation using a reporter construct and found that it was reduced by over-expression of wild-type presenilin 1, but even more reduced in the presence of mutant presenilin 1. These effects on β-catenin and Tcf seem partly to be in contradiction to the findings reported by Zhang *et al.* [2].

Takashima *et al.* [4] have also reported that GSK-3β is in the complex of presenilin 1 and β-catenin, but they additionally found that tau was present in this complex. They found that two mutant forms of presenilin 1 bound more GSK-3β than did wild-type presenilin 1 and that, in cells expressing these two mutant presenilins, co-transfected tau was phosphorylated, whereas in cells expressing wild-type presenilin 1, the co-transfected tau was not phosphorylated. These findings potentially link presenilins, already known to affect amyloid β peptide production, with tau hyperphosphorylation.

What to make of these reports? Well firstly, there now seems little doubt that β-catenin is present in complexes with presenilins in cells, and that GSK-3β is probably also in the same complexes. There is really no dispute over finding that mutant presenilins cause an increase in $A\beta_{42}$ production. An important outstanding issue as far as Alzheimer's disease pathogenesis is concerned is whether mutant presenilins increase GSK-3β activity, perhaps via Notch and forming a closed regulatory loop (Figure 1), which then results in tau hyperphosphorylation and β-catenin destabilisation. Alternatively, some other mechanism may link presenilins to β-catenin metabolism, such as differential binding to wild-type and mutant presenilins and hence sequestration from the cytoplasmic pool. The mechanism is likely to be resolved in the near future, as already many researchers interested in Notch and wingless/Wnt signalling are excited about the possibility of unravelling what's going on in Alzheimer's disease. Perhaps we may then understand how both senile plaques and neurofibrillary tangles are produced in concert.

References

1. Levitan D: **Effects of SEL-12 presenilin on LIN-12 localization and function in *Caenorhabditis elegans*.** *Development* 1998, **125:**3599-3606.
2. Zhang ZH, Hartmann H, Do VM, Abramowski D, Sturchler-Pierrat C, Staufenbiel M, Sommer B, Van de Wetering M, Clevers H, Saftig P, *et al.*: **Destabilization of β-catenin by mutations in presenilin-1 potentiates neuronal apoptosis.** *Nature* 1998, **395:**698-702.
3. Murayama M, Tanaka S, Palacino J, Murayama O, Honda T, Sun XY, Yasutake K, Nihonmatsu N, Wolozin B, Takashima A: **Direct association of presenilin-1 with β-catenin.** *FEBS Lett* 1998, **433:**73-77.
4. Takashima A, Murayama M, Murayama O, Kohno T, Honda T, Yasutake K, Nihonmatsu N, Mercken M, Yamaguchi H, Sugihara S, Wolzonin B: **Presenilin 1 associates with glycogen synthase kinase-3β and its substrate tau.** *Proc Natl Acad Sci USA* 1998, **95:**9637-9641.

5. Yu G, Chen FS, Levesque G, Nishimura M, Zhang DM, Levesque L, Rogaeva E, Xu DH, Liang Y, Duthie M, *et al.*: **The presenilin 1 protein is a component of a high molecular weight intracellular complex that contains β-catenin.** *J Biol Chem* 1998, **273:**16470-16475.
6. Zhou JH, Liyanage U, Medina M, Ho C, Simmons AD, Lovett M, Kosik KS: **Presenilin 1 interaction in the brain with a novel member of the Armadillo family.** *Neuroreport* 1997, **8:**2085-2090.
7. Dale TC: **Signal transduction by the Wnt family of ligands.** *Biochem J* 1998, **329:**209-223.
8. Lovestone S, Reynolds CH: **The phosphorylation of tau: a critical stage in neurodevelopment and neurodegenerative processes.** *Neuroscience* 1997, **78:**309-324.
9. Guo Q, Sopher BL, Furukawa K, Pham DG, Robinson N, Martin GM, Mattson MP: **Alzheimer's presenilin mutation sensitizes neural cells to apoptosis induced by trophic factor withdrawal and amyloid β-peptide: Involvement of calcium and oxyradicals.** *J Neurosci* 1997, **17:**4212-4222.
10. Ahmed Y, Hayashi S, Levine A, Wieschaus E: **Regulation of armadillo by *Drosophila* APC inhibits neuronal apoptosis during retinal development.** *Cell* 1998, **93:**1171-1182.